Hide Items | Restore | Clear | Cancel

DATE: Thursday, April 15, 2004

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT,USOC,EPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L1	10/090326	1
☐	L2	multiplex PCR	911
☐	L3	L1 and first amplification and second amplification	1
☐	L4	L1 and ((first same second) near (amplif$ or cycle$1))	1
☐	L5	L1 and ((different or multiple or plurality) same cycles)	1
☐	L6	L1 and (different temperature or different Tm)	1
☐	L7	multiplex near (PCR or polymerase chain reaction)	981
☐	L8	L7 and (PCR cycles)	173
☐	L9	L8 and (different same (reaction near$3 condition))	0
☐	L10	(PCR or polymerase chain reaction or RT-PCR or reverse transcriptase Polymerase chain reaction or polymerase chain amplification)	75834
☐	L11	L10 AND ((MULTIPLE OR PLURAL$ OR DIFFERENT OR MORE) NEAR CYCLE$1)	2133
☐	L12	L10 AND (SEQUENTIAL NEAR CYCLE$1)	74
☐	L13	L10 AND ((DIFFERENT OR DISTINCT OR SEPARATE) NEAR (TEMPERATURE OR TM))	1817
☐	L14	((l11 OR L12 OR L13) AND ((FIRST NEAR$3 AMPLIFICATION)OR (FIRST NEAR$3 PRIMER SET) OR (FIRST NEAR$3 AMPLICON)))	0
☐	L15	((l11 OR L12 OR L13) AND (FIRST NEAR$3 AMPLIFICATION))	0
☐	L16	((l11 OR L12 OR L13) AND (FIRST NEAR AMPLIFICATION))	206
☐	L17	((l11 OR L12 OR L13) AND (FIRST NEAR PRIEMR SET))	0
☐	L18	((l11 OR L12 OR L13) AND (FIRST NEAR PRIMER SET))	33
☐	L19	((l11 OR L12 OR L13) AND (FIRST NEAR AMPLICON))	21
☐	L20	L19 and (second near amplicon)	7
☐	L21	L18 and (second near primer set)	27
☐	L22	(l16 and (second near amplification))	98
	DB=USPT,DWPI; PLUR=YES; OP=ADJ		
☐	L23	(L18 or l19 or l20 or L21 or l22) and (multiplex or multiplex amplification)	14
	DB=USPT,USOC,EPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L24	GUS near$3 amplicon or 18S-rRNA near$3 amplicon or tyrosinase near$3	0

L28	L27 and (single tube or single vessel or single cartridge)	0
L29	L27 and ((single tube) or (single vessel) or (single cartridge))	0
L30	L25 and ((single tube) or (single vessel) or (single cartridge))	140
L31	L25 same((single tube) or (single vessel) or (single cartridge))	41
L32	((single tube) or (single vessel) or (single cartridge)) same L2	11
L33	L32 and l25	2
L34	L25 and cartridge	464
L35	L34 and compartments	77
L36	L35 and (vessel or reaction vessel)	26
L37	L36 and automat$	16
	DB=PGPB,USPT,USOC,EPAB,DWPI; PLUR=YES; OP=ADJ	
L38	intraoperative PCR	2
L39	(operation or surgery) same (PCR or polymerase chain reaction)	3652
L40	L39 same threshold	37
L41	L40 same indicator transcript	1
L42	((operation or surgery)same patient) same (PCR or polymerase chain reaction)	200
L43	l1 and indicator transcript	1
L44	l43 and CEA transcript	0
L45	l1 and CEA transcript	0
L46	l1 and CEA	1
L47	carcinoembryonic antigen	3294
L48	L47 and l42	21
L49	l48 and (during near$2 (surgery or operation))	0
L50	l48 and (during same(surgery or operation))	9
L51	l42 and (during same(surgery or operation))	79
L52	((PCR or polymerase chain reaction)same (during same(surgery or operation)))	963
L53	L52 same l47	1
L54	L52 same tumor antigen	0
L55	L52 and l47	3
L56	L52 and transcript	61
L57	L52 same transcript	8
L58	l52 same expression	22
L59	l52 same threshold	6
L60	l47 and l7	

END OF SEARCH HISTORY

DOCUMENT-IDENTIFIER: US 5945277 A
TITLE: Strain of hantavirus nucleotide sequences thereof related probes, primers and vectors, and methods for detection

Detailed Description Text (99):
5'-CAGCTGCAGATTATATCTTTAGTGGTTCTTGGTTAGAGATTTCCC-3' (set forth in the Sequence Listing as SEQ ID NO: 11 (each contain a PstI recognition site). The RT-PCR assay (first round) was performed in a single tube reaction containing 5 .mu.l of total RNA (1/10 of isolated RNA, see above) using a GeneAmp PCR System 9600 thermocycler (Perkin Elmer) by heating to 41.degree. C. for 60 min (reverse transcription), followed by 40 cycles of 94.degree. C. for 40 sec, 37.degree. C. for 40 sec, 72.degree. C. for 3 min (amplification); and 72.degree. C. for 5 min (elongation) [final concentrations in 100 .mu.l reaction volume: 1.times. Taq-buffer (Promega), 1.7 mM MgCl.sub.2 (Promega), 0.2 mM dNTPs (Promega), 350 ng of each primer, 11 U Reverse Transcriptase (Boehringer Mannheim), 5 U Taq-polymerase (Promega)]. For the second round 1 .mu.l of first round material was amplified after a denaturing step of 94.degree. C. for 2 min, by 40 cycles of 94.degree. C. for 40 sec, 41.degree. C. for 40sec, and 72.degree. C. for 3 min followed by 72.degree. C. for 5 min. DNA products were extracted with phenol and chloroform, precipitated with ethanol, cut with PstI, and gel-purified using SPIN-X columns (Costar).

DOCUMENT-IDENTIFIER: US 20020111303 A1
TITLE: Diagnostic and therapeutic methods

Detail Description Paragraph:
[0360] We used real time quantitative RT-PCR (Heid, C. A., Stevens, J., Livak, K. J. & Williams, P. M. Real time quantitative PCR. Genome Res. 6, 986-994 (1996); Morrison, T. B., Weis, J. J. & Wittwer, C. T. Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. Biotechniques 24, 954-958 (1998)) to analyse the distribution of BCMP 7 mRNA in normal human tissue mRNAs (Clontech) and breast and prostate cancer cell lines (FIG. 2), and normal breast tissue derived from cosmetic reduction mammoplasties and cancerous breast tissue from surgery (FIG. 3). Ethical approval for the normal and cancer breast samples was obtained at surgery (Oxford, UK). The primers used for PCR were as follows: sense, 5' AGATACCACAGTCAAACCTG 3', antisense, 5' GCACTCATCCAAGTGATGAA 3'. Reactions containing 10 ng cDNA, prepared as described above, SYBR green sequence detection reagents (PE Biosystems) and sense and antisense primers were assayed on an ABI7700 sequence detection system (PE Biosystems). The PCR conditions were 1 cycle at 50.degree. C. for 2 min, 1 cycle at 95.degree. C. for 10 min, and 40 cycles of 95.degree. C. for 15s, 60.degree. C. for 1 min. The accumulation of PCR product was measured in real time as the increase in SYBR green fluorescence, and the data were analysed using the Sequence Detector program v1.6.3 (PE Biosystems). Standard curves relating initial template copy number to fluorescence and amplification cycle were generated using the amplified PCR product as a template, and were used to calculate BCMP 7 copy number in each sample.

DOCUMENT-IDENTIFIER: US 20040053830 A1
TITLE: BCMP84 protein, compositions, diagnostic and therapeutic uses thereof

Detail Description Paragraph:
[0373] Real time RT-PCR was used to quantitatively measure BCMP84 expression in normal human tissue mRNAs (Clontech), breast cancer cell lines, breast cancer tissues removed during surgery, and normal breast tissue removed during breast reduction mammoplasty. Ethical approval for the normal and tumour breast samples was obtained at surgery (University of Oxford, UK). The primers used for PCR were as follows: sense, 5' tctgtgcactctgtcttgga 3' (SEQ ID NO: 6), anti-sense, 5' tagccagctcctctctgtt 3' (SEQ ID NO: 7). Reactions containing 10 ng cDNA, prepared as described above, SYBR green sequence detection reagents (PE Biosystems) and sense and anti-sense primers were assayed on an ABI7700 sequence detection system (PE Biosystems). The PCR conditions were 1 cycle at 50.degree. C. for 2 min, 1 cycle at 95.degree. C. for 10 min, and 40 cycles of 95.degree. C. for 15 sec, 65.degree. C. for 1 min. The accumulation of PCR product was measured in real time as the increase in SYBR green fluorescence, and the data were analyzed using the Sequence Detector program v1.6.3 (PE Biosystems). Standard curves relating initial template copy number to fluorescence and amplification cycle were generated using the amplified PCR product as a template, and were used to calculate BCMP84 copy number in each sample.

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      FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
      ENTERED AT 12:25:56 ON 15 APR 2004
L1          2652 S GODFREY T?/AU OR LUKETICH J?/AU OR PITTSBURGH S?/AU OR FINKEL
L2         13665 S (MULTIPLEX AND AMPLIFICATION) OR (MULTIPLEX POLYMERASE CHAIN
L3             8 S L1 AND L2
L4             2 S L2 AND (DIFFERENT TM OR DIFFERENT TEMPERATURE)
L5             3 S L2 AND ((DIFFERENT OR DISTINCT) (10A) (REACTION CONDITION#))
L6             2 DUP REM L4 (0 DUPLICATES REMOVED)
L7           263 S L2 AND ((DIFFERENT OR MULTIPLE OR DISTINCT OR SEPARATE) AND
L8           134 DUP REM L7 (129 DUPLICATES REMOVED)
L9             2 S L8 AND (FIRST (5A) (PRIMER SET OR AMPLICON OR AMPLIFICATION
L10            0 S L8 AND (SEQUENTIAL (5A) CYCLE#)
L11           19 S L8 AND (PRIMER SET OR AMPLICON)
L12           19 DUP REM L11 (0 DUPLICATES REMOVED)
L13           11 S ((CEA (3A) AMPLICON) OR (TYROSINASE (3A) AMPLICON) OR  (GUS (
L14           19 S L11 AND L2
L15           19 DUP REM L14 (0 DUPLICATES REMOVED)

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TITLE: Homogeneous **multiplex** genotyping of hemochromatosis mutations with fluorescent hybridization probes.
AUTHOR: Bernard P S; Ajioka R S; Kushner J P; Wittwer C T
CORPORATE SOURCE: Department of Pathology, University of Utah Medical School, Salt Lake City 84132, USA.
CONTRACT NUMBER: DK20630 (NIDDK)
GM51647 (NIGMS)
RR00064 (NCRR)
+
SOURCE: American journal of pathology, (1998 Oct) 153 (4) 1055-61.
Journal code: 0370502. ISSN: 0002-9440.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981028

AB **Multiplex polymerase chain reaction amplification** and genotyping by fluorescent probe melting **temperature** (**Tm**) was used to simultaneously detect **multiple** variants in the hereditary hemochromatosis gene. Homogenous real-time analysis by fluorescent melting curves has previously been used to genotype single base mismatches; however, the current method introduces a new probe design for fluorescence resonance energy transfer and demonstrates allele multiplexing by **Tm** for the first time. The new probe design uses a 3'-fluorescein-labeled probe and a 5'-Cy5-labeled probe that are in fluorescence energy transfer when hybridized to the same strand internal to an unlabeled **primer set**. Two hundred and fifty samples were genotyped for the C282Y and H63D hemochromatosis causing mutations by fluorescent melting curves. Multiplexing was performed by including two primer sets and two probe sets in a single tube. In clinically defined groups of 117 patients and 56 controls, the C282Y mutation was found in 87% (204/234) of patient chromosomes, and the relative penetrance of the H63D mutation was 2.4% of the homozygous C282Y mutation. Results were confirmed by restriction enzyme digestion and agarose gel electrophoresis. In addition, the probe covering the H63D mutation unexpectedly identified the A193T polymorphism in some samples. This method is amenable to multiplexing and has promise for scanning unknown mutations.

L12 ANSWER 19 OF 19 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:51543 CAPLUS
DOCUMENT NUMBER: 126:71197
TITLE: Methods and kits for determining pre-**amplification** levels of a nucleic acid target sequence from post-**amplification** levels of product
INVENTOR(S): Ryder, Thomas Brendan; Shannon, Karen W.; Kacian, Daniel Louis; Harvey, Richard C.; McDonough, Sherrol H.; Gonzales, Frank R.; Castillo, Maria R.; Billyard, Elizabeth R.; Shen, Nancy Lau Liu
PATENT ASSIGNEE(S): Gen-Probe Incorporated, USA
SOURCE: Eur. Pat. Appl., 62 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE:

diagnostics

AUTHOR(S): Raja, Siva; El-Hefnawy, Talal; Kelly, Lori A.; Chestney, Melissa L.; **Luketich, James D.**; **Godfrey, Tony E.**

CORPORATE SOURCE: Division of Thoracic Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, 15213, USA

SOURCE: Clinical Chemistry (Washington, DC, United States) (2002), 48(8), 1329-1337

CODEN: CLCHAU; ISSN: 0009-9147

PUBLISHER: American Association for Clinical Chemistry

August 2002

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Rapid-cycling, real-time PCR instruments bring the opportunity for improved intraoperative detection of metastasis to sentinel lymph nodes. Rapid, standardized, and internally controlled assays need to be developed that are sensitive and accurate. The authors describe rapid, multiplexed, internally controlled, quant. reverse transcription-PCR (QRT-PCR) assays for tyrosinase and carcinoembryonic antigen mRNAs on the SmartCycler (Cepheid). A temperature-controlled primer-limiting approach was used to eliminate **amplification** of the endogenous control gene as soon as its signal had reached threshold. Pos.-control oligonucleotide mimics were incorporated into all reactions to differentiate failed reactions from true neg. samples. The optimized assays for rapid QRT-PCR yielded results with threshold cycle values that were only 1-2 cycles higher than slower, more conventional protocols. In rapid PCR, the temperature-controlled **multiplex** assay was quant. over a dynamic range of at least 15 cycles, compared with only 6 cycles for conventional multiplexing methods. All histol. pos. lymph nodes examined were also QRT-PCR pos. for the appropriate marker, and the exogenous, internal pos.-control mimics produced signals in all neg. samples. Internally controlled, rapid QRT-PCR assays can be performed in an intraoperative time frame and with sufficient sensitivity to detect histol. identified metastases to lymph nodes.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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reaction utilizing a primer;
use of a set of DNA primers flanking the Factor-VIII gene on the X-chromosome for hemophilia-A diagnosis

AUTHOR: LIU Q; SOMMER S S
PATENT ASSIGNEE: LIU Q; SOMMER S S
PATENT INFO: US 2002146729 10 Oct 2002
APPLICATION INFO: US 2001-13718 13 Dec 2001
PRIORITY INFO: US 2001-13718 13 Dec 2001; US 1998-113669 24 Jun 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-147576 [14]
AN 2003-07653 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A chromosomal aberration in an individual is detected by performing long distance, **multiplex polymerase chain reaction** (PCR) utilizing a primer.

DETAILED DESCRIPTION - Detection of chromosomal aberrations in an individual comprises performing long distance, **multiplex polymerase chain reaction** (PCR) utilizing a set of primers. The primers are flanking regions of the aberration. The PCR forms a first set of products when performed on a sample from an individual who lacks the aberration. It also forms a second set of products when performed on a sample from an individual who is homozygous or hemizygous for the aberration. It forms a third set of products when performed on a sample from an individual who is heterozygous for the aberration, if the first set of products is produced then the individual does not have the aberration, if the second set of products is produced then the individual is homozygous or hemizygous for the aberration, and if the third set of products is produced then the individual is heterogeneous for the aberration. INDEPENDENT CLAIMS are included for: (a) a method for screening for the presence of hemophilia A in a male comprising performing long distance PCR on a sample from the male using 2 primers which are specific for amplifying a region of an X chromosome comprising an intron 22 of a factor VIII gene under conditions which produce a PCR product when performed on a sample from a person who does not have hemophilia A, where the absence of a PCR product indicates the presence of hemophilia A in the male; (b) a method for performing a PCR comprising cycling the **temperature** between a step of annealing/elongation and a step of denaturation, where the step of annealing/elongation and denaturation is performed at **different** temperatures from each other and where each cycle of PCR comprises a step of denaturation and step of annealing/elongation comprises **temperature** subcycling where the **temperature** is varied at m temperatures of subcycling , where m = at least2 for at least n subcycles, where n = at least1, and where m times n = at least3, for each cycle of PCR where the temperatures of subcycling are lower than a **temperature** required to denature desired PCR products;and (c) a nucleic acid comprising at least13 consecutive bases of a DNA sequence from cDNA sequence of. (i) cDNA sequence tgcccgtcag aagttctcca gcctctacat ct (I); (ii) cDNA sequence ggtcaagact gaaattagcg tgttaggcaaga (II) (iii) cDNA sequence (III) of 1319 base pairs given in the specification; (iv) cDNA sequence of cgaatcacct cccactaggc ccttccttca acag (IV); (v) cDNA sequence of caccgttaga ggagaccagc agcccacaga ct (V); (vi) cDNA sequence (VI) of 695 base pairs given in the specification;(vii) cDNA sequence (VII) of 412 base pairs given in the specification; (viii) cDNA sequence of gccctgcctg tccattacac tgatgacatt atgctgac (VIII); (ix) cDNA sequence of ggccctacaa ccattctgcc tttcactttc agtgcaata (IX); (x) cDNA sequence of cacaagggg aagagtgtga gggtgtggga taagaa (X); (xi) cDNA sequence of

preferred concentration: the PCR comprises dimethyl sulfoxide at a concentration of at least 6%; DNA polymerase at a concentration of at least 0.05 units/microL, and deaza-dGTP at a concentration at least 33% of the concentration of dGTP. Preferred Components: One of the primers comprises cDNA sequence of gcctgcctg tccattacac tgatgacatt atgctgac and another of the primers comprises cDNA sequence of ggcctacaa ccattctgcc tttcactttc agtgcaata. The primers are primers from cDNA sequence of tccattacac tgatgacatt atgctgac and cDNA sequence of tgcccgtcag aagttctcca gcctctacat ct, cDNA sequence of tccattacac tgatgacatt atgctgac and bases 1-36 of cDNA sequence of cccaaacta taaccagcac cttgaacttc ccctctcata or cDNA sequence of cacaaggggg aagagtgtga gggtgtggga taagaa and cDNA sequence of ggccctacaa ccattctgcc tttcactttc agtgcaata.

USE - For determining the presence of chromosomal aberration or abnormalities, e.g., inversions, deletion/inverin and translocations.

ADVANTAGE - The invented method allows for the detection of whether a person is homozygous or hemizygous for the inversion and has hemophilia A. It can achieve efficient and more even **amplification** than normal two or three **temperature** PCR.

EXAMPLE - PCR was performed from human genomic DNA isolated from white blood cells. Three types of cycling conditions were utilized: three-**temperature** PCR, two-**temperature** PCR and S-PCR. The cycling conditions for three-**temperature** PCR were 94 degreesC for 12 seconds, 65 degreesC for 30 seconds and 68 degreesC for 14 minutes for the first 10 cycles. The remaining 20 cycles were performed by adding extra 20 seconds to the elongation per cycle. Conditions for two **temperature** cycling were 94 degreesC for 12 seconds and 64 degreesC for 15 minutes for the first 10 cycles, with an extra 20 seconds added to the elongation per cycles for the remaining 20 cycles. The conditions for S-PCR for the first 10 cycles were 94 degreesC for 12 seconds of annealing/elongation that involved 60 degreesC for 120 seconds and 65 degreesC cycles were modified by addition of extra 3 seconds per cycle. Ten microlL of the reaction was mixed with loading buffer and incubated at 37 degreesC for 5 minutes. Samples were electrophoresized on 0.6% agarose gel, and stained with ethidium bromide for ultraviolet photography.(29 pages)

L12 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER:	2002:158022 CAPLUS
DOCUMENT NUMBER:	136:178967
TITLE:	Rapid single-tube screening of the C282Y hemochromatosis mutation by real-time **multiplex** allele-specific PCR for diagnosis of disease
INVENTOR(S):	Kairisto, Veli; Donohoe, Gerard; Eskola, Jarkko; Korpela, Timo
PATENT ASSIGNEE(S):	Finland
SOURCE:	PCT Int. Appl., 32 pp. CODEN: PIXXD2
DOCUMENT TYPE:	Patent
LANGUAGE:	English
FAMILY ACC. NUM. COUNT:	1
PATENT INFORMATION:	

product

INVENTOR(S): Ryder, Thomas Brendan; Shannon, Karen W.; Kacian, Daniel Louis; Harvey, Richard C.; McDonough, Sherrol H.; Gonzales, Frank R.; Castillo, Maria R.; Billyard, Elizabeth R.; Shen, Nancy Lau Liu

PATENT ASSIGNEE(S): Gen-Probe Incorporated, USA

SOURCE: Eur. Pat. Appl., 62 pp.
CODEN: EPXXDW

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 747488	A1	19961211	EP 1996-107602	19960513
EP 747488	B1	20030709		
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE				
US 5705365	A	19980106	US 1995-482428	19950607
US 5710029	A	19980120	US 1995-486705	19950607
CA 2223241	AA	19961219	CA 1996-2223241	19960502
WO 9640990	A1	19961219	WO 1996-US6300	19960502
W: AU, CA, JP, KR				
AU 9656748	A1	19961230	AU 1996-56748	19960502
AU 709292	B2	19990826		
JP 11506013	T2	19990602	JP 1996-500512	19960502
AT 244770	E	20030715	AT 1996-107602	19960513

PRIORITY APPLN. INFO.:
US 1995-482428 A 19950607
US 1995-486705 A 19950607
WO 1996-US6300 W 19960502

AB Methods and kits are provided for relating initial amts. of target nucleic acids present in a sample to target-specific **amplification** products. The transcription-mediated **amplification** system is capable of producing a quant. relationship between target input and target-specific output. Transcription-based **amplification** includes any of a variety of isothermal **amplification** methods which utilize RNA transcription to achieve nucleic acid **amplification**; two enzymes, a reverse transcriptase derived from a retrovirus such as Moloney murine leukemia virus, and an RNA polymerase (e.g., from bacteriophage T7), are used in conjunction with one or more primers having a 5' sequence encoding a promoter sequence. Further, methods are described for carefully controlling this relationship resulting in an unexpectedly high degree of reproducibility. Also described are useful methods for extending the dynamic range of transcription-based **amplification** systems. Thus, reaction conditions are made submaximal by reducing the concentration of a single enzyme, RNA polymerase, in the reaction mixture such that the amount of target in the starting sample can be determined with a high degree of accuracy and with high sensitivity over a reproducible dynamic range in a **multiplex amplification** format (simultaneous **amplification** of more than one **different amplicon**). In a single **amplicon** format, the reproducible dynamic quantifiable range of the **amplification** can be extended to 6-8 logs, with a precision of between 0.3 and 0.2 logs or less. Furthermore, <10 initial copies of the target sequence can be quantified. Altering the promoter sequence of the promoter-primer through the introduction of one or more base substitutions or deletions to reduce the number of RNA transcripts produced by the RNA

levels. Lowering the reaction **temp**. by as little as 4°
can cause 100-1000-fold less product to be made under otherwise identical
conditions, and the Mg2+ concentration also modulates the extent of the
amplification reactions. Quantitation of HIV-1 viral RNA and
hepatitis B virus DNA demonstrate the utility of the transcription-based
amplification system.

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author]; Sobsey, M. D. [Reprint author]
CORPORATE SOURCE: University of North Carolina, Chapel Hill, NC, USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology (2002) Vol. 102, pp. 441-442. print.
Meeting Info.: 102nd General Meeting of the American Society for Microbiology. Salt Lake City, UT, USA. May 19-23, 2002. American Society for Microbiology.
ISSN: 1060-2011.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Dec 2002
Last Updated on STN: 4 Dec 2002

AB A fast and quantitative method for specific detection of waterborne viruses would permit a rapid response and implementation of control measures. Most waterborne viruses are RNA viruses detectable by reverse transcription-PCR (RT-PCR). Fluorescent monitoring through real-time RT-PCR detection offers fast, quantitative, sensitive and specific method. This study compares **multiplex** Taqman RT-PCR and SYBR Green I RT-PCR **amplification** for simultaneous detection of hepatitis A virus (HAV) and Coxsackievirus B3 (CVB3, a representative enterovirus). A rapid, simple and inexpensive RT-PCR assay was developed using two pairs of primers with SYBR Green I dye and no probe for the simultaneous detection of HAV and/or enterovirus with real-time monitoring of the **amplification** process in the LightCycler (Roche, Indianapolis, IN). Using **multiple** sequence alignments, a pair of primers was chosen which amplifies all types of human enterovirus, and is compatible with probes for the Taqman assay. HAV and CVB3 were **amplification** yielded amplicons of 244 and/or 145 bp respectively, and these amplicons were detectable by melting curve analysis in the SYBR green RT-PCR assay with **Tm** of 79degreeC and 84degreeC, respectively. Simultaneous detection of HAV and enterovirus with Taqman RT-PCR and the Smart Cycler (Cepheid, Sunnyvale, CA) was attempted using HAV and CVB3 **amplicon** -specific internal Taqman probes with FAM and ROX at 5'end, respectively. The detection limits were 2.4 and 3.2 PFU/PCR reaction for HAV and CVB3, respectively. Using positive control synthetic HAV RNA templates, as few as 10 genome equivalents were detected. A linear relationship between threshold cycle (CT) and logarithm concentration of RNA templates was observed. Overall, HAV and enterovirus genomes were simultaneously detected in a single tube by Taqman RT-PCR in 90 min with automation. Application of the method to field samples of virus concentrates from water is now needed to evaluate detection of low levels of enteric viruses in source and finished drinking waters and in recreational and shellfishing waters.

L15 ANSWER 12 OF 19 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-04910 BIOTECHDS
TITLE: Analysis of polynucleotides in a sample using generic capture sequences comprises amplifying target polynucleotide, and utilizing the product to indirectly assay the sample for the polynucleotide;
DNA **amplification** useful for pharmacogenetics, forensics, anthropology, paternity testing, functional genomics, genetic analysis, SNP, immunoglobulin mutation, pathogen detection, drug resistance, DNA sequencing anddiagnosis
AUTHOR: LAI J H; PHILLIPS V E; WATSON A R
PATENT ASSIGNEE: QUANTUM DOT CORP
PATENT INFO: WO 2001

AN 2002-04910 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Assaying, (M1) for an **amplification** product (AMP) from a first target polynucleotide, (TP), comprising providing a sample that is suspected of containing AMP, where the AMP is a polynucleotide comprising a first label and a capture sequence not present in the TP at the same position, is new.

DETAILED DESCRIPTION - A target polynucleotide, (TP), is amplified, where first primer has a tag sequence, the complement of which is formed on the opposite strand during **amplification** and is referred to as capture sequence (CS), and the opposite strand is referred to as an **amplification** product (AMP) has a label; probe conjugated to substrate specific to CS, is contacted with AMP to form AMP detection complex. Assaying (M1) for an AMP from a first TP, comprises providing a sample that is suspected of containing AMP, where the AMP is a polynucleotide comprising a first label and a capture sequence not present in the TP at the same position, where the AMP is formed by primer extension from a template, where the template comprises a complement to the TP and a target noncomplementary region, where the capture sequence is a complement to the target noncomplementary region, providing a substrate that is conjugated to a first capture probe, contacting the sample with the capture probe under a first set of hybridization conditions, where the capture probe can bind to the capture sequence under the first set of hybridization conditions and determining if the first label is associated with the substrate. INDEPENDENT CLAIMS are also included for the following: (1) forming (M2) an AMP detection complex for assaying a sample for a first TP; (2) an **amplification** product detection complex comprising a capture probe polynucleotide hybridized to capture sequence of labeled **amplification** product from a TP, where the capture probe polynucleotide is conjugated to a substrate, where the capture sequence is not present in a region of the TP which is amplified and is introduced into the **amplification** product by copying a template polynucleotide, the template polynucleotide comprising a target noncomplementary region and a region complementary to the TP, where the target noncomplementary region was introduced into the template polynucleotide by extension of a primer hybridized to the target polynucleotide, the primer comprising the target noncomplementary region and where the capture sequence is complementary to the target noncomplementary region; and (3) a kit for assaying for an AMP from a TP comprises a substrate attached to a capture probe, a first primer comprising a 3' end a first target complementary region located at the 3' end of the first primer, and a first target noncomplementary region that is not complementary to the first TP at a position 3' of a sequence to which the first target complementary region can hybridize, a second primer, label, housing for retaining the substrate, first primer, second primer, and a label and instruction provided with the housing that describe how to use the components of the kit to assay a sample by forming an AMP from the TP using the first and second primers that comprises the label and a capture sequence that is complementary to the target noncomplementary in the first primer, where the capture sequence can bind to the capture probe.

BIOTECHNOLOGY - Preferred Method: In (M1) the capture probe is a polynucleotide, substrate is from micorsphere, chip, slide, multiwell plate, a membrane, an optical fiber, and an optionally porous gel matrix, more preferably slide. The substrate is preferably conjugated to several **different** capture probe polynucleotides having corresponding **different** sequences, where each of the **different** capture probes can selectively bind to a corresponding **different**

GaAs, GaSb, InN, InP, InAs, InSb, AlAs, AlP, AlS, Ge, Si, Pb, PObSe, PbSe, their alloy or their mixture, more preferably the core is a CdSe; (b) a shell, preferably ZnS. The AMP is produced by a process comprising incorporation of a nucleotide comprising the first label into AMP using a polymerase or extension of a primer using a polymerase to form AMP, where primer comprises the first label, where the first label comprises an agent selected from chromophore; lumiphore; fluorophore preferably semiconductor nanocrystal, fluorescent dye, lanthanide chelate, a green fluorescent protein, more preferably is a lanthanide chelate selected from europium chelate, terbium chelate and a samarium chelate; chromogen; hapten; antigen; radioactive isotope; magnetic particle; metal nanoparticle; enzyme preferably alkaline phosphatase, horseradish peroxidase, beta-galactosidase, glucose oxidase, bacterial luciferase, an insect luciferase and sea pansy luciferase; antibody or binding portion or their equivalent; aptamer; and one member of a binding pair; or an agent selected from avidin, streptavidin, digoxigenin and biotin. The method preferably comprises determining if the first label, preferably a fluorophore, is associated with the substrate comprises applying a light source to the substrate that can excite the fluorophore, and determining if a fluorescence emission from the fluorophore occurs from the substrate. The sample is preferably assayed for the presence of AMP or to determine its amount, where AMP is produced at a detectably higher level from at least one allele of a locus having at least two alleles. When the substrate is a first microsphere, (M1) further comprises assaying the sample for containing a second, third and fourth AMP from a second, third and fourth TP, where second AMP from second TP and is further contacted under a second set of hybridization conditions with a second capture probe conjugated to a second microsphere, where the second capture probe is a polynucleotide, the second microsphere can be the first microsphere or a **different** second microsphere, the second microsphere is a **different** second microsphere comprising a second spectral code comprising second fluorescent characteristics, the second spectral code distinguishable from the first spectral code, the second capture probe can hybridize to the second AMP under the second set of hybridization conditions, the second AMP comprises a second label, which can be the first label when the second microsphere is a **different** second microsphere or can be a **different** second label, and determining if the second label is associated with the second microsphere, where the first and the second AMPs are produced from a single locus, or differ by a single nucleotide. The substrate is further conjugated to a second/third/fourth capture probe, where the second/third/fourth capture probe can preferentially bind to a second/third/fourth sequence on a second/third/fourth AMP, second/third/fourth comprising a second/third/fourth second/third/fourth label that can be the same as or **different** than the first label and/or the second label where the binding the second/third/fourth AMP to the second/third/fourth capture probe can be independently determined. In (M2), after altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product, the sample is further contacted with the first and second primers under conditions in which the first and second primers can hybridize to the second and first primer extension products, respectively, and be extended to form several first and second primer extension products, where altering the sample conditions to allow dissociation of the first and second primer extension product from the first target polynucleotide and first primer extension product, respectively comprises heating the sample. (M2) further comprising removing single-stranded polynucleotides from the sample prior to altering the sample conditions to allow dissociation of the second primer